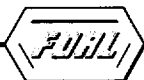


FOOD AND DRUG

LABORATORIES, INC.



MAURICE AVENUE AT 58TH STREET, MASPETH, NEW YORK 11378

February 15, 1972

Teratologic Evaluation of FDA 71-1

(Ammonium glycyrrhizinate)

in

Mice, Rats, Hamsters and Rabbits

Final report-Teratologic Evaluation of FDA 71-1 (Ammonium Glycyrrhizinate) in
Mice, Rats, Hamsters & Rabbits 2/15/72

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M I C E

Food and Drug Research Laboratories
INCORPORATED



Maurice Avenue at 58th Street
Maspeth, New York 11378

Telephone: TWining 4-0800

Cable: Foodlabs, New York

FINAL
REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date: February 15, 1972

Laboratory No. 0728 b
Contract No. FDA 71-260

Sample: Fine dark brown powdered material.

Marking: FDA 71-1 (Ammonium glycyrrhizinate)

Examination Requested: Teratologic evaluation of FDA 71-1 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

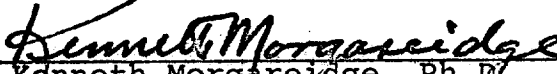
Conclusion:

Attention is called to the fact that this is the first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 1000 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or no maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.


Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 1 through 6

Material: FDA 71-1

Table 1
Fate Summary
(Mice)

Date February 15, 1972

Laboratory No. 0728 b

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
1	Sham	0	27	20	25	19
2	Aspirin*	200	30	26	20	14
3	FDA 71-1	27	28	20	24	19
4	FDA 71-1	90	30	24	27	23
5	FDA 71-1	300	30	24	25	22
6	FDA 71-1	1000	25	21	24	21

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 1 through 6

Date February 15, 1972

Material: FDA 71-1

Table 2

Laboratory No. 0728 b

Reproduction Data
(Mice)

Group:	1	2	3	4	5	6
Dose (mg/kg):	Sham	Aspirin*	27	90	300	1000
Number of females:						
Total pregnant	20	26	20	24	24	21
Pregnant at term	19	14	19	23	22	21
Number of live litters:	20	14	19	23	22	21
Number of implant sites:						
Total	247	308	251	289	283	273
Average/pregnant dam	12.4	11.8	12.6	12.0	11.8	13.0
Number of fetuses alive:						
Total	240	165	221	252	252	260
Average/live litter	12.0	11.8	11.6	11.0	11.5	12.4
Average/pregnancy at term	12.6	11.8	11.6	11.0	11.5	12.4
Number of fetuses dead:						
Total	5	49	3	10	9	2
Litters with one or more dead	4	6	2	9	3	2
Pregnancies at term (%)	21.1	42.9	10.5	39.1	13.6	9.5
Litters with all dead	0	4	0	0	1	0
Pregnancies at term (%)	0	28.6	0	0	4.5	0
Number of resorptions:						
Total:	2	94	27	27	9	11
Litters with one or more resorptions	1	12	9	8	8	8
Pregnancies at term (%)	5.3	85.7	47.4	34.8	36.4	38.1
Litters with total resorptions	0	8	1	1	0	0
Pregnancies at term (%)	0	57.1	5.3	4.3	0	0
Average fetus weight, g.	0.97	0.87	0.93	0.93	0.99	0.98

* Positive control at 200 mg/kg

FOOD and DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Table 3

Laboratory No. 0728b

Material FDA 71-1

Summary of Skeletal Findings
(Mice)

Date February 15, 1972

Findings	Group No: 1	2	3	4	5	6
	Dose (mg/kg): Sham	Aspirin	27	90	300	1000
		**				
<u>Live Fetuses Examined</u>	163/19	115/14	155/19	186/23	179/22	181/21
<u>Fetuses with:</u>						
Incomplete sternebrae	14/6	20/7	58/18	81/22	24/12	45/14
Scrambled sternebrae				1/1		10/1
Bipartite sternebrae	123/17	75/14	110/19	151/23	91/19	106/18
Missing sternebrae						
Fused sternebrae						
Extra sternebrae	3/3	1/1				
Incomplete hyoid						5/3
<u>Fetuses with:</u>						
Scrambled vertebrae						
Tail defects (short, scrambled, etc.)	1/1					
Incomplete ossification; vertebrae	137/17	57/10	87/13	99/18	66/15	81/18
<u>Fetuses with:</u>						
Fused ribs				3/1		
Incomplete ribs		6/1	1/1			
Wavy ribs	1/1					
Less than 12 ribs						
More than 13 ribs	2/2	5/2	5/3	6/3		5/3
<u>Other Findings:</u>						
Scoliosis						
Delayed cranial ossification	25/7	32/9	11/5	17/5	16/5	41/9
Craniostosis						
Feet; retarded ossification	115/16	35/4	97/11	87/15	73/11	156/19
Meningocele			1/1	1/1		

* Numerator = Number of fetuses affected; Denominator = number of litters affected

200 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Date February 15, 1972

Species Mice

Table 4

Laboratory No. 0728 b

Average Body Weights *

Group	Material	Dose Level mg/kg	-----Day-----				
			0	6	11	15	17**
			-----g-----				
1	Sham	0	29.3	31.4	34.6	44.1	49.6 (19)
2	Aspirin	200	28.8	31.0	31.1	36.1	44.8 (14)
3	FDA 71-1	27	29.0	31.4	34.7	43.6	48.7 (19)
4	FDA 71-1	90	29.7	31.8	35.6	41.9	49.8 (23)
5	FDA 71-1	300	29.6	32.5	35.9	45.2	50.9 (22)
6	FDA 71-1	1000	30.4	32.7	36.5	45.9	52.2 (21)

* Of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1).



Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 1

Appendix II

Date February 15, 1972

Material Sham

Reproduction Data in Mice (Individual)

Laboratory No. 0728

Dose 0

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 8001	P	18	18	0	0	0.91	
S 8002	P	12	12	0	0	0.89	
S 8003	P	15	15	0	0	0.63	
S 8004	NP	0				--	Died Day 16
S 8005	P	14	12	2	0	1.05	
S 8006	P	14	14	0	0	0.96	
S 8007	P	14	14	0	0	1.03	
S 8008	P	9	9	0	0	0.79	
S 8009	P	11	11	0	0	1.31	
S 8010	P	12	11	1	0	0.95	
S 8011	P	13	13	0	0	0.89	
S 8012	NP	0				--	
S 8013	P	10	10	0	0	0.87	
S 8014	NP	0				--	
S 8015	P	13	13	0	0	0.85	
S 8016	P	15	15	0	0	0.75	
S 8017	P	11	11	0	0	1.00	
S 8018	P	12	11	1	0	0.81	
S 8019	P	9	9			--	Dam died day 14
S 8020	P	16	14	0	2	0.98	
S 8021	NP	0				--	
S 8022	NP	0				--	
S 8023	NP	0				--	
S 8024	NP	0				--	
S 8025	P	6	6	0	0	1.53	
S 8026	P	13	13	0	0	1.15	
S 8027	P	10	9	1	0	1.12	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 2

Appendix II

Date February 15, 1972

Material Aspirin

Reproduction Data in Mice (Individual)

Laboratory No. 0728

Dose 200 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 8001	P	12	0	12	0	--	Died day 15.
A 8002	P	10	10	0	0	0.74	
A 8003	NP	0				--	
A 8004	P	12	0	0	12	--	Died day 16.
A 8005	P	7	0	0	7	--	
A 8006	P	2	0	0	2	--	
A 8007	P	14	13	0	1	0.80	
A 8008	P	13	0	0	13	--	Died day 12.
A 8009	P	11	0	0	11	--	Died day 17.
A 8010	P	16	0	0	16	--	Died day 12.
A 8011	P	13	10	0	3	0.69	
A 8012	P	13	0	0	13	--	Died day 11.
A 8013	P	14	0	0	14	--	Died day 14.
A 8014	P	13	13	0	0	0.74	
A 8015	P	12	0	12	0	--	Died day 9.
A 8016	P	12	11	0	1	0.92	
A 8017	P	15	0	15	0	--	Died day 10.
A 8018	P	12	12	0	0	1.11	
A 8019	P	8	0	8	0	--	Died day 16
A 8020	NP	0				--	
A 8021	P	12	11	1	0	0.81	
A 8022	NP	0				--	
A 8023	P	14	14	0	0	0.83	
A 8024	P	11	11	0	0	1.08	
A 8025	P	8	8	0	0	0.82	
A 8026	P	14	14	0	0	0.88	
A 8027	NP	0				--	
A 8028	P	15	14	1	0	0.89	
A 8029	P	12	12	0	0	1.03	
A 8030	P	13	12	0	1	0.87	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 3

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Mice (Individual)

Laboratory No. 0728 b

Dose 27 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 8001	P	13	13	0	0	1.00	
B 8002	P	11	11	0	0	0.93	
B 8003	P	15	12	2	1	1.06	
B 8004	P	9	9	0	0	0.86	
B 8005	P	11	11	0	0	--	
B 8006	P	16	15	1	0	--	
B 8007	P	13	13	0	0	0.99	
B 8008	NP	0				--	
B 8009	P	15	13	0	2	1.02	
B 8010	NP	0	0	0	0	--	Died day 13.
B 8011	P	12	11	0	1	0.97	
B 8012	P	15	15	0	0	1.15	
B 8013	P	9	9	0	0	0.94	
B 8014	P	14	14	0	0	0.72	
B 8015	NP	0				--	
B 8016	P	12	10	0	2	0.76	
B 8017	P	12	10	0	2	0.89	
B 8018	P	10	9	0	1	0.92	
B 8019	NP	0				--	
B 8020	P	11	10	0	1	0.89	
B 8021	NP	0				--	Died day 12.
B 8022	NP	0				--	Died day 10.
B 8023	NP	0				--	
B 8024	NP	0				--	
B 8025	P	12	12	0	0	0.98	
B 8026	P	12	12	0	0	1.01	
B 8027	P	14	12	0	2	0.78	
B 8028	P	15	0	0	15	--	Died day 10.

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 4

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Mice (Individual)

Laboratory No. 0728 b

Dose 90 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 8031	P	15	13	0	2	0.97	
B 8032	P	13	12	1	0	0.88	
B 8033	P	11	11	0	0	0.91	
B 8034	NP	0				--	
B 8035	P	15	7	0	8	1.06	
B 8036	P	16	16	0	0	0.95	
B 8037	P	12	12	0	0	1.01	
B 8038	NP	0				--	
B 8039	NP	0				--	Died day 12.
B 8040	NP	0				--	Died day 14.
B 8041	P	9	8	1	0	0.79	
B 8042	P	10	10	0	0	0.89	
B 8043	P	15	14	1	0	0.74	
B 8044	P	10	10	0	0	1.15	
B 8045	P	14	13	0	1	0.82	
B 8046	P	14	13	0	1	1.00	
B 8047	P	11	3	2	6	0.74	
B 8048	P	13	12	1	0	0.75	
B 8049	P	8	7	1	0	0.87	
B 8050	P	13	12	1	0	1.04	
B 8051	P	11	11	0	0	1.07	
B 8052	NP	0				--	
B 8053	NP	0				--	
B 8054	P	12	11	1	0	1.10	
B 8055	P	14	11	0	3	0.93	
B 8056	P	10	10	0	0	0.89	
B 8057	P	14	13	0	1	0.87	
B 8058	P	5	0	0	5	--	Died day 17.
B 8059	P	13	12	1	0	0.96	
B 8060	P	11	11	0	0	1.08	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 5

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Mice (Individual)

Laboratory No. 0728 b

Dose 300 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 8061	P	11	11	0	0	0.85	
B 8062	P	3	3	0	0	1.17	
B 8063	NP	0				--	Died day 14.
B 8064	NP	0	/			--	Died day 14.
B 8065	NP	0				--	Died day 7.
B 8066	P	13	11	0	2	0.88	
B 8067	P	14	14	0	0	0.86	
B 8068	NP	0				--	
B 8069	P	11	10	0	1	1.05	
B 8070	P	15	15	0	0	0.96	
B 8071	P	12	11	0	1	0.85	
B 8072	P	14	14	0	0	1.04	
B 8073	P	12	11	0	1	0.86	
B 8074	P	15	15	0	0	1.09	
B 8075	P	11	11	0	0	1.14	
B 8076	P	14	14	0	0	0.91	
B 8077	P	2	2	0	0	0.96	
B 8078	P	15	11	2	2	0.97	
B 8079	P	8	8	0	0	1.05	
B 8080	P	14	13	0	1	0.98	
B 8081	P	6	0	6	0	--	Died day 10.
B 8082	NP	0				--	
B 8083	P	14	14	0	0	1.04	
B 8084	NP	0				--	
B 8085	NP					--	Died day 8.
B 8086	P	10	10	0	0	1.14	
B 8087	P	14	13	0	1	1.07	
B 8088	P	15	15	0	0	0.98	
B 8089	P	15	14	0	1	0.85	
B 8090	P	12	12	0	0	0.99	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 6

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Mice (Individual)

Laboratory No. 0728 b

Dose 1000 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 8091	NP	0				--	
B 8092	P	13	12	1	0	1.01	
B 8093	P	16	15	0	1	0.96	
B 8094	--	--	/				Not assigned.
B 8095	--	--					Not assigned.
B 8096	P	12	12	0	0	1.22	
B 8097	P	15	13	0	2	0.87	
B 8098	P	14	11	0	3	0.89	
B 8099	P	17	17	0	0	0.81	
B 8100	P	13	12	0	1	1.15	
B 8101	P	14	14	0	0	0.77	
B 8102	NP	0				--	Died day 15.
B 8103	P	13	13	0	0	0.96	
B 8104	P	11	10	0	1	0.94	
B 8105	P	11	10	0	1	0.87	
B 8106	P	12	12	0	0	1.08	
B 8107	NP	0				--	
B 8108	P	11	11	0	0	1.00	
B 8109	P	10	10	0	0	0.98	
B 8110	P	11	11	0	0	0.96	
B 8111	P	13	12	0	1	0.95	
B 8112	P	14	13	0	1	1.05	
B 8113	P	13	12	1	0	0.95	
B 8114	P	14	14	0	0	0.87	
B 8115	P	12	12	0	0	1.28	
B 8116	NP	0				--	
B 8117	P	14	14	0	0	0.95	

* P= Pregnant; NP= Not Pregnant

R A T S

Food and Drug Research Laboratories
I N C O R P O R A T E D



Maurice Avenue at 58th Street
Maspeth, New York 11378

Telephone: TWining 4-0800

Cable: Foodlabs, New York

FINAL
R E P O R T

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date: February 15, 1972

Laboratory No. 0729b
Contract No. FDA 71-260

Sample: Fine dark brown powdered material.

Marking: FDA 71-1 (Ammonium glycyrrhizinate).

Examination Requested: Teratologic evaluation of FDA 71-1 in rats.

Procedure: See Appendix I.

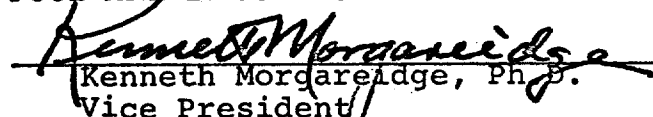
Results: See Tables 1 through 4 and Appendix II.

Conclusion: Attention is called to the fact that this is the first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 1000 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.


Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 1 through 6

Material: FDA 71-1

Table 1

Fate Summary
(Rats)

Date February 15, 1972

Laboratory No. 0729 b

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
1	Sham	0	24	21	25	21
2	Aspirin *	250	26	20	22	19
3	FDA 71-1	27	24	22	24	21
4	FDA 71-1	90	26	20	23	20
5	FDA 71-1	300	25	21	21	18
6	FDA 71-1	1000	25	22	21	20

* Positive Control

Group: 1 through 6

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Date February 15, 1972Material: FDA 71-1

Table 2

Laboratory No. 0729 bReproduction Data
(Rats)

Group:	1	2	3	4	5	6
Dose (mg/kg):	Sham	Aspirin*	27	90	300	1000
Number of females:						
Total pregnant	21	20	22	20	21	22
Pregnant at term	21	19	21	20	18	20
Number of live litters:	21	19	21	20	21	21
Number of implant sites:						
Total	245	230	220	218	254	254
Average/pregnant dam	11.7	11.5	10.0	10.9	12.1	11.5
Number of fetuses alive:						
Total	242	206	202	199	246	236
Average/live litter	11.5	10.8	9.62	9.95	11.7	11.2
Average/pregnancy at term	11.5	10.8	9.62	9.95	13.7	11.8
Number of fetuses dead:						
Total	0	9	0	0	1	11
Litters with one or more dead	0	1	0	0	1	2
Pregnancies at term (%)	0	5.26	0	0	5.56	10.0
Litters with all dead	0	0	0	0	0	1
Pregnancies at term (%)	0	0	0	0	0	5.0
Number of resorptions:						
Total:	3	15	18	19	7	7
Litters with one or more resorptions	3	8	11	8	4	6
Pregnancies at term (%)	14.3	42.1	52.4	14.0	22.2	30.0
Litters with total resorptions	0	0	1	0	0	0
Pregnancies at term (%)	0	0	4.8	0	0	0
Average fetus weight, g.	4.05	3.55	4.02	3.98	3.64	3.73

* Positive control at 250 mg/kg

FOOD and DRUG RESEARCH LABORATORIES, INC.

Laboratory No. 0729 b

Date February 15, 1972

Table 3

Summary of Skeletal Findings
(Rats)

Findings	Group No: 1	2	3	4	5	6
	Dose (mg/kg): Sham	Aspirin**	27	90	300	1000
<u>Live Fetuses Examined</u>	173/21	148/19	152/21	143/20	149/18	160/20
<u>Fetuses with:</u>						
Incomplete sternebrae	101/19	106/19	97/20	90/19	105/17	115/20
Scrambled sternebrae		2/1		2/2	2/2	
Bipartite sternebrae	7/6	5/5	4/4	6/5	3/3	4/4
Missing sternebrae	39/12	58/14	27/8	24/9	48/17	32/12
Fused sternebrae			8/5			
Extra sternebrae						
<u>Fetuses with:</u>						
Scrambled vertebrae						
Tail defects (short, scrambled, etc.)						
<u>Fetuses with:</u>						
Fused ribs						
Incomplete ribs	1/1	1/1	1/1	1/1		1/1
Wavy ribs	8/6	24/12	11/6	4/3	15/8	20/11
Less than 12				2/1		
<u>Other Findings:</u>						
Scoliosis						
Delayed cranial ossification	20/10	29/11	12/8	10/8	15/7	11/7
Craniostosis		1/1			2/2	
Incomplete ossification of phalanges		2/2	1/1		2/2	1/1

* Numerator = Number of fetuses affected; Denominator = number of litters affected

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Species Rats

Table 4*

Average Body Weights

Date February 15, 1972

Laboratory No. 0729 b

Group	Material	Dose Level mg/kg	-----Day-----				
			0	6	11	15	20**
			-----g-----				
1	Sham	0	240.5	253.0	265.8	281.3	360.2 (21)
2	Aspirin	250	240.1	251.6	258.8	280.1	335.8 (19)
3	FDA 71-1	27	241.1	247.3	257.4	277.0	337.0 (22)
4	FDA 71-1	90	239.3	248.3	261.8	277.7	337.2 (20)
5	FDA 71-1	300	237.9	247.2	253.7	273.0	343.6 (18)
6	FDA 71-1	1000	239.8	250.5	259.1	275.5	344.1 (20)

* Of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1).



Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 1

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rats (Individual)

Laboratory No. 0729

Dose Sham

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 9001	P	10	9		1	3.81	
S 9002	P	10	10			4.11	
S 9003	NP	0				--	
S 9004	P	11	11			5.86	
S 9005	P	10	10			3.65	
S 9006	P	12	12			4.17	
S 9007	P	16	16			4.16	
S 9008	P	11	11			3.87	
S 9009	P	12	12			4.93	
S 9010	P	12	12			3.56	
S 9011	P	14	14			3.87	
S 9012	P	15	15			3.88	
S 9013	P	12	12			3.71	
S 9014	P	10	10			3.37	
S 9015	P	9	8		1	4.02	
S 9016	P	10	10			3.89	
S 9017	P	13	13			3.68	
S 9018	P	6	6			3.57	
S 9019	P	13	13			3.45	
S 9020	P	13	12		1	3.72	
S 9021	NP	0				--	
S 9022	P	14	14			6.07	
S 9023	P	12	12			3.80	
S 9024	NP	0				--	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 2

Appendix II

Date February 15, 1972

Material Aspirin

Reproduction Data in Rats (Individual)

Laboratory No. 0729

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 9001	NP	0				--	Died Day 16.
A 9002	NP	0				--	Died Day 19.
A 9003	P	8	8			3.52	
A 9004	NP	0				--	Died Day 15
A 9005	NP	0				--	
A 9006	P	10	10			3.29	
A 9007	P	11	11			3.37	
A 9008	P	15	14		1	3.39	
A 9009	P	10	8		2	3.49	
A 9010	P	14	14			3.13	
A 9011	P	11	11			5.13	
A 9012	P	12	11		1	3.27	
A 9013	P	16	15		1	3.48	
A 9014	NP	0				--	
A 9015	P	12	12			3.48	
A 9016	P	11		9	2	--	Died Day 19.
A 9017	P	11	11			3.70	
A 9018	P	14	11		3	3.59	
A 9019	P	13	13			3.77	
A 9020	P	12	12			3.62	
A 9021	P	13	13			3.20	
A 9022	NP	0				--	
A 9023	P	10	10			3.39	
A 9024	P	8	7		1	3.41	
A 9025	P	5	1		4	3.57	
A 9026	P	14	14			3.73	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 3

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rats (Individual)

Laboratory No. 0729 b

Dose 27 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 9001	P	14	14			3.79	
B 9002	P	11	11			3.64	
B 9003	NP	0				--	
B 9004	P	7	6		1	5.59	
B 9005	P	10	9		1	4.05	
B 9006	P	8	8			4.03	
B 9007	P	11	11			3.41	
B 9008	P	11	10		1	4.00	
B 9009	P	12	11		1	3.65	
B 9010	P	8	7		1	3.67	
B 9011	P	13	13			3.54	
B 9012	P	9	8		1	5.59	
B 9013	NP	0				--	
B 9014	P	11	9		2	3.83	
B 9015	P	9	8		1	4.18	
B 9016	P	12	11		1	3.86	
B 9017	P	10	10			3.35	
B 9018	P	16	16			3.37	
B 9019	P	12	12			3.81	
B 9020	P	6	3		3	3.38	
B 9021	P	12	12			4.08	
B 9022	P	1	1			6.40	
B 9023	P	12	12			3.14	
B 9024	P	5			5	--	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 4

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rats (Individual)

Laboratory No. 0729 b

Dose 90 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 9031	P	12	10		2	3.54	
B 9032	P	17	17			3.96	
B 9033	NP	0				--	Died Day 20.
B 9034	P	11	4		7	3.75	
B 9035	NP	0				--	
B 9036	P	11	11			4.01	
B 9037	P	14	10		4	3.65	
B 9038	P	12	12			3.81	
B 9039	P	13	12		1	3.82	
B 9040	NP	0				--	
B 9041	P	9	9			4.05	
B 9042	P	11	11			3.63	
B 9043	P	7	6		1	4.15	
B 9044	P	14	13		1	3.67	
B 9045	NP	0				--	Died Day 12.
B 9046	P	13	12		1	3.63	
B 9047	P	9	9			3.96	
B 9048	P	11	11			4.13	
B 9049	P	14	14			3.93	
B 9050	NP	0				--	Died Day 14.
B 9051	P	6	6			3.57	
B 9052	NP	0				--	
B 9053	P	6	6			6.87	
B 9054	P	11	11			4.06	
B 9055	P	7	5		2	3.79	
B 9056	P	10	10			3.69	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 5

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rats (Individual)

Laboratory No. 0729 b

Dose 300 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 9061	P	11	10		1	3.46	
B 9062	NP	0				--	Died Day 17.
B 9063	P	9	9			4.36	
B 9064	NP	0				--	
B 9065	P	11	11			--	Died Day 8.
B 9066	NP	0				--	
B 9067	P	11	11			3.59	
B 9068	P	12	12			3.46	
B 9069	P	11	8	1	2	3.17	
B 9070	P	14	14			3.24	
B 9071	P	13	13			3.51	
B 9072	P	12	12			3.23	
B 9073	NP	0				--	
B 9074	P	11	11			3.80	
B 9075	P	11	8		3	3.54	
B 9076	P	11	11			3.63	
B 9077	P	14	14			4.06	
B 9078	P	10	9		1	3.72	
B 9079	P	12	12			3.55	
B 9080	P	15	15			--	Died Day 14.
B 9081	P	11	11			3.78	
B 9082	P	17	17				Died Day 8.
B 9083	P	15	15			3.94	
B 9084	P	12	12			3.28	
B 9085	P	11	11			4.21	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 6

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rats (Individual)

Laboratory No. 0729 b

Dose 1000 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 9091	P	12	12			3.99	
B 9092	P	15	14		1	3.79	
B 9093	P	10	10			3.75	
B 9094	P	9	9			3.94	
B 9095	NP	0				--	Died Day 10.
B 9096	P	14	14			3.86	
B 9097	P	10	10			3.80	
B 9098	P	9	7		2	3.58	
B 9099	P	10	10			3.75	
B 9100	P	15	15			3.39	
B 9101	P	12	11		1	3.77	
B 9102	P	12	12			3.55	
B 9103	NP	0				--	
B 9104	P	7	7			3.56	
B 9105	P	11	11			3.75	
B 9106	P	13	13			3.34	
B 9107	NP	0				--	Died Day 10.
B 9108	P	14	13		1	3.62	
B 9109	P	7	7			4.20	
B 9110	P	12	12			3.54	
B 9111	P	12	11		1	4.16	
B 9112	P	10		10		--	Died Day 20.
B 9113	P	13	12	1		3.70	
B 9114	P	14	14			--	Died Day 13.
B 9115	P	13	12		1	3.50	

* P = Pregnant; NP = Not Pregnant

HAMSTERS



FINAL
R E P O R T

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date: February 15, 1972

Laboratory No. 0730 b
Contract No. FDA 71-260

Sample: Fine dark brown powdered material.

Marking: FDA 71-1 (Ammonium glycyrrhizinate).

Examination Requested: Teratologic evaluation of FDA 71-1 in hamsters.

Procedure: See Appendix I.


Results: See Tables 1 through 4 and Appendix II.

Conclusion: Attention is called to the fact that this is the first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 1000 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.


Kenneth Morgareidge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 1 through 6

Material: FDA 71-1

Table 1

Fate Summary
(Hamsters)

Date February 15, 1972

Laboratory No. 0730 b

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
1	Sham	0	23	22	23	21
2	Aspirin*	250	24	21	24	21
3	FDA 71-1	27	24	21	24	21
4	FDA 71-1	90	24	22	24	22
5	FDA 71-1	300	24	23	24	23
6	FDA 71-1	1000	25	23	25	23

* Positive Control

Group: 1 through 6

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Date February 15, 1972Material: FDA 71-1

Table 2

Laboratory No. 0730 bReproduction Data
(Hamsters)

Group:	1	2	3	4	5	6
Dose (mg/kg):	Sham	Aspirin*	27	90	300	1000
Number of females:						
Total pregnant	22	21	21	22	23	23
Pregnant at term	21	21	21	22	23	23
Number of live litters:	21	21	21	22	23	23
Number of implant sites:						
Total	281	262	275	297	297	309
Average/pregnant dam	12.8	12.5	13.1	13.5	12.9	14.0
Number of fetuses alive:						
Total	264	257	265	287	279	298
Average/live litter	12.6	12.2	12.6	13.0	12.1	13.0
Average/pregnancy at term	12.6	12.2	12.6	13.0	12.1	13.0
Number of fetuses dead:						
Total	0	1	0	0	3	1
Litters with one or more dead	0	1	0	0	3	1
Pregnancies at term (%)	0	4.8	0	0	13.0	4.3
Litters with all dead	0	0	0	0	0	0
Pregnancies at term (%)	0	0	0	0	0	0
Number of resorptions:						
Total:	17	4	10	10	15	10
Litters with one or more resorptions	5	3	7	5	9	9
Pregnancies at term (%)	23.8	14.3	33.3	22.7	39.1	39.1
Litters with total resorptions	1	0	0	0	0	0
Pregnancies at term (%)	4.8	0	0	0	0	0
Average fetus weight, g.	1.72	1.81	1.87	1.80	1.80	1.80

* Positive control at 250 mg/kg

FOOD and DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Table 3

Laboratory No. 0730 b

Material FDA 71-1

Summary of Skeletal Findings
(Hamsters)

Date February 15, 1972

Findings	Group No:	1	2	3	4	5	6
	Dose (mg/kg):	Sham	Aspirin**	27	90	300	1000
<u>Live Fetuses Examined</u>		189/21	184/21	192/21	202/22	207/23	213/23
<u>Fetuses with:</u>							
Incomplete sternebrae		17/6	20/10	27/13	35/13	28/17	19/7
Scrambled sternebrae							
Bipartite sternebrae		115/19	91/19	122/21	143/21	123/19	150/22
Missing sternebrae							
Fused sternebrae					1/1		
Extra sternebrae				1/1	8/5		3/3
Incomplete hyoid							
<u>Fetuses with:</u>							
Scrambled vertebrae							
Tail defects (short, scrambled, etc.)							
Incomplete ossification: Vertebrae		54/12	82/17	92/18	91/18	90/18	97/16
<u>Fetuses with:</u>							
Fused ribs		1/1				1/1	
Incomplete ribs		5/4	1/1		26/14	3/3	6/5
Wavy ribs							
Less than 12 ribs							
More than 13 ribs			1/1		16/7	15/8	10/7
<u>Other Findings:</u>							
Scoliosis							
Delayed cranial ossification		17/2	10/2	1/1	19/8	23/6	100/14
Craniostosis					1/1	1/1	
Feet; retarded ossification		9/2	1/1	6/5		27/4	6/2

* Numerator = Number of fetuses affected; Denominator = number of litters affected

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Species Hamsters

Table 4

Average Body Weights *

Date February 15, 1972

Laboratory No. 0730 b

Group	Material	Dose Level mg/kg	-----Day-----				
			0	6	8	10	14**
1	Sham	0	106.2	110.2	113.7	124.7	144.8 (21)
2	Aspirin	250	104.9	109.3	112.6	121.8	144.8 (21)
3	FDA 71-1	27	110.6	116.4	120.1	132.4	153.5 (21)
4	FDA 71-1	90	111.0	115.5	118.1	131.4	153.8 (22)
5	FDA 71-1	300	109.7	113.3	116.3	127.7	149.2 (23)
6	FDA 71-1	1000	110.7	115.3	119.9	131.6	154.3 (23)

* of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1).



Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation; the controls were sham-treated.

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 15, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of skeletal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 1

Appendix II

Date February 15, 1972

Material Sham

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 0001	P	12	12			1.62	
S 0002	P	12	12			1.82	
S 0003	P	9	9			1.66	
S 0004	P	14	14			2.01	
S 0005	P	17	15		2	1.97	
S 0006	P	14	14			2.06	
S 0007	P	10	10			1.94	
S 0008							Number not assigned.
S 0009	P	14	12		2	1.85	
S 0010	P	12	12			1.03	
S 0011	P	13	13			1.78	
S 0012	P	16	15		1	1.63	
S 0013	P	14	13		1	1.88	
S 0014	P	14	14			1.80	
S 0015	P	17	17			2.00	
S 0016	P	10	10			0.98	
S 0017	P	15	15			1.70	
S 0018	P	10	10			1.65	
S 0019	P	16	16			1.84	
S 0020	P	12	12			1.68	
S 0021	NP	0				--	
S 0022	P	10	10			1.77	
S 0023	P	9	9			1.55	
S 0024	P	11			11	--	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 2

Appendix II

Date February 15, 1972

Material Aspirin

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 0001	P	13	13			1.75	
A 0002	NP	0				--	
A 0003	P	13	13			1.82	
A 0004	P	12	11	1		2.12	
A 0005	P	12	12			1.90	
A 0006	NP	0				--	
A 0007	P	15	15			2.10	
A 0008	NP	0				--	
A 0009	P	13	13			1.67	
A 0010	P	12	12			1.04	
A 0011	P	14	14			1.91	
A 0012	P	11	11			1.67	
A 0013	P	11	10		1	1.94	
A 0014	P	14	13		1	1.82	
A 0015	P	16	16			1.80	
A 0016	P	12	12			1.61	
A 0017	P	13	13			1.89	
A 0018	P	13	13			1.87	
A 0019	P	12	12			1.74	
A 0020	P	12	12			1.84	
A 0021	P	14	14			1.62	
A 0022	P	11	11			1.69	
A 0023	P	10	8		2	1.86	
A 0024	P	9	9			2.42	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 3

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730 b

Dose 27 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 0001	P	13	13			1.69	
B 0002	P	12	12			1.93	
B 0003	P	16	16			1.94	
B 0004	P	12	11		1	1.94	
B 0005	NP	0				--	
B 0006	P	13	13			1.99	
B 0007	P	13	13			1.85	
B 0008	P	14	14			1.91	
B 0009	P	11	11			2.83	
B 0010	P	9	8		1	1.45	
B 0011	P	12	11		1	1.82	
B 0012	NP	0				--	
B 0013	P	15	15			1.78	
B 0014	P	14	14			1.97	
B 0015	P	15	15			2.00	
B 0016	P	12	10		2	1.96	
B 0017	P	14	13		1	1.81	
B 0018	P	13	13			1.94	
B 0019	NP	0				--	
B 0020	P	13	13			1.56	
B 0021	P	14	13		1	1.85	
B 0022	P	12	12			1.78	
B 0023	P	13	13			1.72	
B 0024	P	15	12		3	1.49	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 4

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730 b

Dose 90 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 0031	P	12	12			1.71	
B 0032	P	13	13			1.98	
B 0033	P	12	12			1.86	
B 0034	P	16	16			1.70	
B 0035	NP	0				--	
B 0036	NP	0				--	
B 0037	P	9	9			2.05	
B 0038	P	19	17		2	1.93	
B 0039	P	14	13		1	1.61	
B 0040	P	14	14			2.07	
B 0041	P	15	14		1	1.70	
B 0042	P	16	16			1.82	
B 0043	P	12	12			1.78	
B 0044	P	11	11			1.82	
B 0045	P	13	9		4	1.80	
B 0046	P	15	15			1.84	
B 0047	P	17	17			1.70	
B 0048	P	13	13			1.87	
B 0049	P	12	12			1.80	
B 0050	P	11	11			1.85	
B 0051	P	12	12			1.72	
B 0052	P	13	13			1.48	
B 0053	P	14	14			1.62	
B 0054	P	14	12		2	1.96	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 5

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730 b

Dose 300 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 0061	P	12	12			1.92	
B 0062	P	12	12			1.92	
B 0063	P	17	14		3	1.77	
B 0064	P	16	16			1.92	
B 0065	P	15	15			1.82	
B 0066	P	9	8		1	1.87	
B 0067	P	12	12			1.77	
B 0068	P	12	11		1	1.90	
B 0069	P	11	11			1.08	
B 0070	P	17	14		3	1.80	
B 0071	P	13	13			2.08	
B 0072	P	9	7	1	1	1.82	
B 0073	P	11	11			1.81	
B 0074	P	16	16			2.00	
B 0075	P	13	12	1		1.72	
B 0076	P	15	15			1.55	
B 0077	P	11	10	1		1.81	
B 0078	P	13	13			2.01	
B 0079	P	10	9		1	1.63	
B 0080	P	16	15		1	1.74	
B 0081	P	12	9		3	1.89	
B 0082	P	14	13		1	1.83	
B 0083	NP	0				--	
B 0084	P	11	11			1.70	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 6

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Hamsters (Individual)

Laboratory No. 0730 b

Dose 1000 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
B 0091	P	12	11		1	1.92	
B 0092	P	13	13			2.08	
B 0093	P	15	15			2.02	
B 0094	P	11	10		1	1.55	
B 0095	P	13	13			1.98	
B 0096	P	12	12			1.98	
B 0097	P	16	16			1.56	
B 0098	P	16	16			1.86	
B 0099	P	14	13		1	0.89	
B 0100	P	15	15			2.04	
B 0101	NP	0				--	
B 0102	P	12	12			1.73	
B 0103	P	14	14			1.77	
B 0104	P	12	11		1	1.94	
B 0105	P	15	13		2	1.71	
B 0106	P	12	12			1.72	
B 0107	P	16	15	1		1.75	
B 0108	P	13	13			1.94	
B 0109	P	14	14			1.94	
B 0110	P	15	14		1	1.79	
B 0111	P	13	12		1	1.72	
B 0112	P	11	10		1	1.79	
B 0113	NP	0				--	
B 0114	P	14	13		1	1.74	
B 0115	P	11	11			1.88	

* P = Pregnant; NP = Not Pregnant

RABBITS

Food and Drug Research Laboratories
INCORPORATED



Maurice Avenue at 58th Street
Maspeth, New York 11378

Telephone: TWining 4-0800

Cable: Foodlabs, New York

FINAL
REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date: February 15, 1972

Laboratory No. 0731 b
Contract No. FDA 71-260

Sample: Fine dark brown powdered material.

Marking: FDA 71-1 (Ammonium glycyrrhizinate).

Examination Requested: Teratologic evaluation of FDA 71-1 in rabbits.

Procedure: See Appendix I.

Results: See Tables 1 through 4 and Appendix II.

Conclusion: Attention is called to the fact that this is the first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 1000 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge
Kenneth Morgareidge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 1 through 6

Material: FDA 71-1

Table 1

Fate Summary
(Rabbits)

Date February 15, 1972

Laboratory No. 0731 b

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
1	Sham	0	15	10	13	8
2	6-AN *	5	15	10	14	4
3	FDA 71-1	27	15	12	15	12
4	FDA 71-1	90	15	10	15	10
5	FDA 71-1	300	15	11	14	10
6	FDA 71-1	1000	15	10	10	7

* Positive Control: 6 - Amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 1 through 6

Date February 15, 1972

Material: FDA 71-1

Table 2
Reproduction Data
(Rabbits)

Laboratory No. 0731 b

Group:	1	2	3	4	5	6
Dose (mg/kg):	Sham	6-AN *	27	90	300	1000
Number of females:						
Total pregnant	10	10	12	10	11	10
Pregnant at term	8	4	12	10	10	7
Number of corpora lutea:						
Total	106	87	111	89	96	75
Average/pregnant dam	10.6	8.7	9.25	8.9	8.7	7.5
Number of live litters:	6	4	12	10	10	7
Number of implant sites:						
Total	39	30	70	52	56	46
Average/pregnant dam	3.9	3.0	5.8	5.2	5.1	4.6
Number of fetuses alive:						
Total	14	8	64	42	48	38
Average/pregnant dam	1.40	0.80	5.3	4.2	4.4	3.8
Average/live litter	2.33	2.0	5.3	4.2	4.8	5.4
Average/pregnancy at term	1.75	2.0	5.3	4.2	4.8	5.4
Number of fetuses dead:						
Total	12	1	0	5	0	6
Litters with one or more dead	3	1	-	2	-	2
Pregnancies at term (%)	37.5	25.0	-	40.0	-	28.5
Litters with all dead	2	0	-	1	-	1
Pregnancies at term (%)	25.0	-	-	10.0	-	14.2
Number of resorptions:						
Total:	13	25	6	5	8	2
Litters with one or more resorptions	7	9	6	4	3	1
Pregnancies at term (%)	87.5	>100	50.0	40.0	30.0	14.2
Litters with total resorptions	2	6	0	0	1	0
Pregnancies at term (%)	25.0	>100	-	-	10.0	-
Average fetus weight, g.	40.5	30.0	34.9	34.9	40.3	39.4

* Positive control: 5.0 mg/kg 6 amino nicotinamide dosed on Day 9.

FOOD and DRUG RESEARCH LABORATORIES, INC.

Laboratory No. 0731 b

Date February 15, 1972

Table 3

Summary of Skeletal Findings
(Rabbits)Groups 1 through 6
Material FDA 71-1

Findings	Group No:	1	2	3	4	5	6
	Dose (mg/kg):	Sham	6-AN**	27	90	300	1000
<u>Live Fetuses Examined</u>		14/6	8/4	64/12	45/10	48/10	34/7
<u>Fetuses with:</u>							
Incomplete sternebrae		7/3	4/3	25/10	20/7	12/6	4/3
Scrambled sternebrae			2/2	2/2	1/1	1/1	2/2
Bipartite sternebrae			2/2			1/1	
Missing sternebrae			1/1	1/1	1/1		
Fused sternebrae			2/2	6/4		1/1	3/2
Extra sternebrae							
<u>Fetuses with:</u>							
Scrambled vertebrae							
Tail defects (short, scrambled, etc.)							
Club foot			2/2				
<u>Fetuses with:</u>							
Fused ribs			2/2				
Incomplete ribs		2/2	2/2	4/2	8/5	10/6	1/1
Wavy ribs				1/1			
Less than 12			1/1				
<u>Other Findings:</u>							
Scoliosis							
Delayed cranial ossification							
Craniostosis		6/3	4/2	15/7	5/5	8/4	
Craniobifida				1/1			

* Numerator = Number of fetuses affected; Denominator = number of litters affected

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 1 through 6

Date February 15, 1972

Species Rabbits

Table 4

Laboratory No. 0731 b

Average Body Weights *

Group	Material	Dose Level mg/kg	Day-----				
			0	6	12	18	29**
			----- kg -----				
1	Sham	0	2.39	2.39	2.36	2.31	2.31 (8)
2	6-AN***	5	2.34	2.43	2.27	----	2.09 (9)
3	FDA 71-1	27	2.41	2.41	2.40	2.32	2.40 (12)
4	FDA 71-1	90	2.52	2.53	2.51	2.51	2.49 (10)
5	FDA 71-1	300	2.34	2.35	2.34	2.33	2.34 (10)
6	FDA 71-1	1000	2.26	2.38	2.33	2.23	2.60 (7)

* Of pregnant dams.

** Number of surviving dams in parentheses (c.f. Table 1).

*** 6-amino nicotinamide dosed on Day 9.



Appendix I

Teratology Study in Rabbits

Virgin, adult, Dutch-belted female rabbits were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. On Day 0, each doe was given an injection of 0.4 ml of human chorionic gonadotropin (400 IU) via the marginal ear vein. Three hours later, each doe was inseminated artificially with 0.3 ml of diluted semen from a proven donor buck using approximately 20×10^6 motile sperm according to the procedure described by Vogin et al (Pharmacologist 11, 282 (1969)). Beginning on Day 6 and continuing daily through Day 18 the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 12, 18, and 29 of gestation. All animals were observed daily for appearance and behavior, with particular attention to food consumption and body weight in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 29 all does were subjected to Caesarean section under surgical anesthesia, and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were recorded. Body weights of the live pups were also recorded. The urogenital tract of each animal was examined in detail for normality. In addition all fetuses underwent a detailed gross examination for the presence of external congenital abnormalities. The live fetuses of



each litter were then placed in an incubator for 24 hours for the evaluation of neonatal survival. All surviving pups were sacrificed, and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 1

Appendix II

Date February 15, 1972

Material Sham

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731

Dose 0

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
S 1001	P	13	3	3			38.4	
S 1002	P	6	2	1		1	36.1	
S 1003	NP	0	0				---	
S 1004	NP	4	0				---	
S 1005	P	9	6	5		1	40.3	
S 1006	P	13	3	2		1	43.9	
S 1007	P	7	7		7		---	Died day 20.
S 1008	NP	0	0				---	
S 1009	P	5	4		4		---	Died day 10.
S 1010	NP	0	0				---	
S 1011	P	4	2			2	---	
S 1012	P	17	5	1	1	3	40.7	
S 1013	P	13	4	2		2	43.7	
S 1014	P	5	3			3	---	
S 1015	NP	10	0				---	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 2
6- Amino
 Material nicotinamide
 Dose 5 mg/kg

Appendix II

Date February 15, 1972

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
Z 1001	P	2	2			2	---	
Z 1002	P	5	2			2	---	
Z 1003	P	5	4			4	---	
Z 1004	P	3	1			1	---	
Z 1005	P	9	5	1	1	3	24.1	1 Neonatal death.
Z 1006	NP	4	0				---	
Z 1007	P	10	2	1		1	20.1	1 Neonatal death.
Z 1008	P	6	4			4	---	
Z 1009	NP	5	0				---	
Z 1010	NP	4	0				---	
Z 1011	P	4	0			4	---	
Z 1012	P	12	4	4			38.6	
Z 1013	P	10	6	2		4	37.3	2 Neonatal deaths.
Z 1014	NP	5	0				---	Died day 22.
Z 1015	NP	3	0				---	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 3

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731 b

Dose 27 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
B 1001	P	6	4	4			32.4	4 Neonatal deaths.
B 1002	P	7	6	6			38.1	1 Neonatal death.
B 1003	P	10	4	3		1	38.7	
B 1004	P	13	8	8			26.3	
B 1005	P	10	6	6			38.5	
B 1006	P	9	4	3		1	34.9	
B 1007	P	8	8	8			31.6	1 Neonatal death.
B 1008	P	11	7	6		1	32.4	
B 1009	P	8	6	6			28.8	
B 1010	P	4	2	1		1	42.1	
B 1011	P	8	8	7		1	32.1	1 Neonatal death.
B 1012	NP	0	0				---	
B 1013	NP	3	0				---	Pyometra.
B 1014	NP	0	0				---	
B 1015	P	14	7	6		1	43.3	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 4

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731 b

Dose 90 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
B 1016	P	12	9	9			27.0	5 Neonatal deaths.
B 1017	P	5	5	5			40.6	
B 1018	P	4	4	4			44.6	
B 1019	P	12	8	5	1	2	30.7	
B 1020	P	7	5		4	1	30.5	
B 1021	NP	0	0				---	No ovaries.
B 1022	NP	8	0				---	
B 1023	P	5	4	3		1	39.3	
B 1024	NP	0	0				---	
B 1025	P	7	7	7			34.4	
B 1026	NP	0	0				---	
B 1027	P	8	6	6			34.9	
B 1028	P	13	1	1			33.3	
B 1029	P	8	3	2		1	34.1	
B 1030	NP	0	0				---	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 5

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731 b

Dose 300 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
B 1031	NP	0	0				---	
B 1032	P	11	5	5			42.8	
B 1033	NP	0	0				---	
B 1034	P	7	6	6			42.5	
B 1035	P	17	4	4			41.0	
B 1036	P	7	4	4			37.7	
B 1037	NP	0	0				---	
B 1038	P	6	3	3			42.3	
B 1039	P	6	2	2			42.0	
B 1040	P	11	7	6		1	33.8	
B 1041	P	10	8	8			41.2	1 Neonatal death.
B 1042	NP	0	0				---	
B 1043	P	7	6			6	---	Aborted Day 28.
B 1044	P	4	4	3		1	37.7	
B 1045	P	10	7	7			42.1	1 Neonatal death.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 6

Appendix II

Date February 15, 1972

Material FDA 71-1

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731 b

Dose 1000 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
B 1046	NP	0	0	-			---	
B 1047	NP	0	0				---	
B 1048	P	10	2	2			54.6	1 Neonatal death.
B 1049	P	8	7	7			45.0	
B 1050	P	10	10	10			36.1	
B 1051	NP	0	0				---	
B 1052	P	7	2	2			23.2	Died Day 19.
B 1053	P	6	3	3			---	Died Day 18; Dosing mishap.
B 1054	P	7	3		1	2	---	Aborted Day 21.
B 1055	P	5	5		5		---	Aborted Day 22.
B 1056	NP	0	0				---	Died Day 26.
B 1057	NP	0	0				---	
B 1058	P	10	9	9			29.9	
B 1059	P	7	2	2			40.0	
B 1060	P	5	3	3			47.4	

* P = Pregnant; NP = Not Pregnant